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## A STUDY OF THE CHROMOSOMES OF HIPPONOË ESCULENTA AND MOIRA ATROPOS.

EDITH PINNEY.

The comparatively recent investigations made on fertilized echinoderm eggs with especial regard to the question of chromosome individuality had their beginning in an attempt to correlate the behavior of the chromosomes during fertilization and the subsequent cleavage stages with the observed facts of parental dominance in hybrid cultures.

The first observations are recorded by Tennent, '07. He figures the equatorial plates of *Toxopneustes variegatus*, *Moira atropos* and *Arbacia punctulata* in which individual characteristics for each species are evident. The contrast between the chromosome groups in *Moira* and *Arbacia* is so apparent that in the equatorial plates of the hybrid  $\frac{\textit{Arbacia} \text{ } \sigma}{\textit{Moira} \text{ } \text{f}}$  the hybrid nature of the segmentation nucleus is easily recognized.

Baltzer, '09, in an extensive morphological study of the chromosomes of the European forms, *Strongylocentrotus lividus* and *Echinus microtuberculatus*, was able to identify single chromosomes which could be recognized in the hybrid  $\frac{\textit{Strongylocen-}}{\textit{Echinus} \text{ } \text{f}}$   
 $\textit{trotus} \text{ } \sigma$ .<sup>1</sup> The main facts which he describes may for the sake of comparison be briefly stated at this point.

The chromosomes of *Strongylocentrotus* are in general rod-shaped bodies which vary in length. The longest are over three times the length of the shortest. In addition to these there are in one-half of the eggs two and in the other half, three hook-shaped elements. The occasional third hook is smaller than the other two which are equal in size and evidently form a somatic

<sup>1</sup> Boveri in his "Zellen Studien," Heft VI., 1907, Jena, called attention to Baltzer's observations which were being made at that time in Boveri's laboratory. He publishes one figure of *Strongylocentrotus* showing a hook-shaped chromosome. He himself in 1890 had noted the occurrence of rods of different lengths in certain echinoderms.

pair. In all of the eggs the somatic number of chromosomes is the same; *i. e.*, thirty-six. In accordance with previous conclusions upon the occurrence of the heterochromosomes in insects, the dimorphism in the chromosome groups of *Strongylocentrotus* is correlated with the dimorphism of sex in echinoderms. However, Baltzer, from his observations on *Echinus* eggs cross-fertilized with *Strongylocentrotus* sperm, together with observations on multipolar spindles in disperm eggs, concludes that the odd hook is originally present in the egg. Instead of two kinds of sperm as, we know, exist in the insects, in the echinoderms there are two kinds of eggs. To quote from Wilson, '10, "it is the female that is the heterogametic sex while the male is homogametic. . . . Sex production in these animals must therefore conform to the formulas,

Egg  $F$  + Spermatozoan  $G$  =  $FG$ , Female.

Egg  $G$  + Spermatozoan  $G$  =  $GG$ , Male."

Tennent has obtained evidence which, I think, shows that these formulas do not hold for all echinoderms. An account of his interesting observations appears elsewhere in this journal.

In *Echinus*, which also contains thirty-six chromosomes, the occurrence of three characteristic pairs was established; viz., a pair of large hooks, a pair of small horse-shoe or V-shaped chromosomes and a pair of very long rods. A small V-shaped unpaired chromosome is also found in one half of the eggs which corresponds to the unpaired hook in *Strongylocentrotus*.

In a later paper, '10, Baltzer describes characteristic elements in the chromosome groups of *Sphærechinus granularis* and *Arbacia pustulosa*. The number of somatic chromosomes in *Sphærechinus* is forty-two. All are rod-shaped. Two somatic pairs could however be distinguished in all of the eggs by reason of their excessive lengths. No odd chromosome could be demonstrated in this species due, no doubt, to the lack of differentiation in form of the members of the group. *Arbacia* showed a number of small hooks and U-shaped chromosomes but the unfavorable nature of the egg prevented detailed study. The somatic number is forty. The chromosomes in this species resemble those of *Arbacia punctulata* figured by Tennent, '07, in that they are as a whole comparatively short.

In 1910, before the appearance of Baltzer's later paper, Miss Heffner published observations made in this laboratory upon *Toxopneustes variegatus*, one of the American species which has proved so valuable in cross breeding experiments.<sup>1</sup> She found in straight fertilized *Toxopneustes* eggs a somatic pair of long rods approximating in length the long rods of *Sphærechinus*. Half of the eggs contain two and half three V-shaped elements. The unpaired V is undoubtedly the accessory chromosome. The chromosome number in *Toxopneustes* is thirty-six, the same as in *Strongylocentrotus* and *Echinus*. Miss Heffner also examined *Arbacia punctulata* but found the eggs unfavorable for detailed cytological study, a fact which forms another point of resemblance between the American and European species described.

The present paper deals with two additional species of echinoids, *Hipponoë esculenta* (*Tripneustes esculentus*) and *Moira atropos*. My special object has been to determine whether any or all of the chromosomes exhibit any of the various expressions of individuality which have been reported for the other forms mentioned above.

I am indebted to Dr. Tennent, at whose instance the work was undertaken, for the material used and direction during the course of its investigation. I wish also to express my gratitude to Dr. Stevens for her interest and helpful suggestions.

### *Hipponoë esculenta.*

The *Hipponoë* material was collected and preserved at the Tortugas laboratory of the Carnegie Institution in the summers of 1909 and 1910 by Dr. Tennent. It was prepared in the usual manner for study, the only stain used being Heidenhain's iron-hæmatoxylin. Observations made on first and second cleavage spindles form the chief basis of my conclusions.

The character of the *Hipponoë* spindle delayed somewhat the solution of the problem at hand. The cytoplasm of the egg is beautifully clear and the chromosomes compared to other echinoderm chromosomes are of good size, but during division they are so crowded on the spindle that they obscure one another. This may be attributed to several causes. It may be due to the

<sup>1</sup> Tennent, '07, '10a, '10b and '11.

thickness of the chromosomes which is a specific character. Figs. 1a, 1b and 25 show equatorial plates of *Hipponoë* and *Moirā* respectively, and by comparing them one sees that the *Hipponoë* rods are thicker than those of *Moirā*. It may be that the spindle radius is less in *Hipponoë* than in other forms. Again an attractive force may exist among the elements of one daughter group and this might cause clumping.

There are many reasons why the anaphase stages are most favorable for studies of this sort but there is no doubt that much can be gained from a study of equatorial plates. As will be seen from the figures referred to, the polar views of metaphase stages are especially suitable for determining the relative thickness of the chromosomes, although as Tennent, '07, has pointed out, the contrast is rarely great enough to justify the exclusive use of this character as a means of distinguishing between the chromosomes of two species in hybrids. The segments of the contracted spireme in metaphase have not yet been influenced by the spindle fibers or the force which produces division, whatever that may be, and therefore show a uniform thickness. Such equatorial plates are composed of rod-shaped chromosomes of varying lengths. They may be straight, curved or even bent at various angles. In the latter case it could not be determined whether the resulting V-like chromosomes were to be identified with the V's found in the division stages. Neither is it possible to determine the length or number of the rods. Crowding and overlapping in some parts of the plate prevents the separation of the elements or their correct measurement. If however the chromosomes maintain constant size relations, as we should expect from the abundance of evidence on this point gained from forms more suitable for its demonstration, and since, as I have shown in my figures, the thickness of the rods in this stage is constant, it seems that the relative lengths of the chromosomes at this stage must be constant also and that in the subsequent division stages where the thickness of the rods does evidently vary the length relations vary also. This I consider a point of great importance since it is on just such a comparison of the lengths of the chromosomes in division stages that Baltzer, '10, depends for much of his evidence as to the identity of the elimi-

nated chromosomes in the hybrids. Baltzer makes no comparison between the equatorial plates of the species that he studied. In his figures of anaphases of different species the daughter chromosomes are shown as rods of equal thickness. He regards the apparent variations in the thickness of the rods in sections stained with Heidenhain's iron-hæmatoxylin as the fault of the stain and his conclusions as to the uniform thickness of the rods are drawn from observations made upon aceto-carmine preparations. One would naturally expect to find the rods which lie farthest from the surface of the section retaining the stain longest. The deeper lying rods might then appear thicker but all of the rods lying in one optical plane would show the same width. All of the rods in one optical plane, however, are not of the same width. It might be argued that a disturbance in the section after staining or variations in the thickness of the sections would produce this effect but the variations in width are of too frequent occurrence to be ascribed to any such causal factors.

I am convinced from my own observations that the rods of one spindle not only vary in thickness as they are drawn toward the poles but they vary irregularly. The explanation is simple. During division the proximal ends of the two halves of a rod are drawn toward opposite poles while the distal ends are still united. This latter opposing force results in the lengthening of the two daughter chromosomes during their separation. As soon as the separation is complete the rods begin to contract. Since some rods are much longer than others the accomplishment of their separation and their subsequent contraction would naturally be delayed and their relative lengths in a late anaphase would be more apparent than real.

For illustration I refer to Baltzer's later paper, '10, Figs. 1, *a* and *b*. In these he shows a *Strongylocentrotus* spindle with only two rod-shaped chromosomes over eight mm. long. The two spindles shown in his Figs. 4, *a* and *b*, and 12, *a* and *b* from the same species each show three rods which exceed eight mm. On the other hand Figs. 5, *a* and *b*, show thirteen and Figs. 6, *a* and *b*, ten chromosomes from one spindle which are more than eight mm. long.

Again Figs. 5, *a* and *b*, show one chromosome over 15 mm.

long while the next longest measures only a trifle over 11  $\mu$ m. If as we would naturally suppose, the two longest in this case form a somatic pair, it is demonstrated that the force which causes stretching during division and contraction after the daughter elements have separated, may work very irregularly indeed and that the chromosomal lengths taken from these



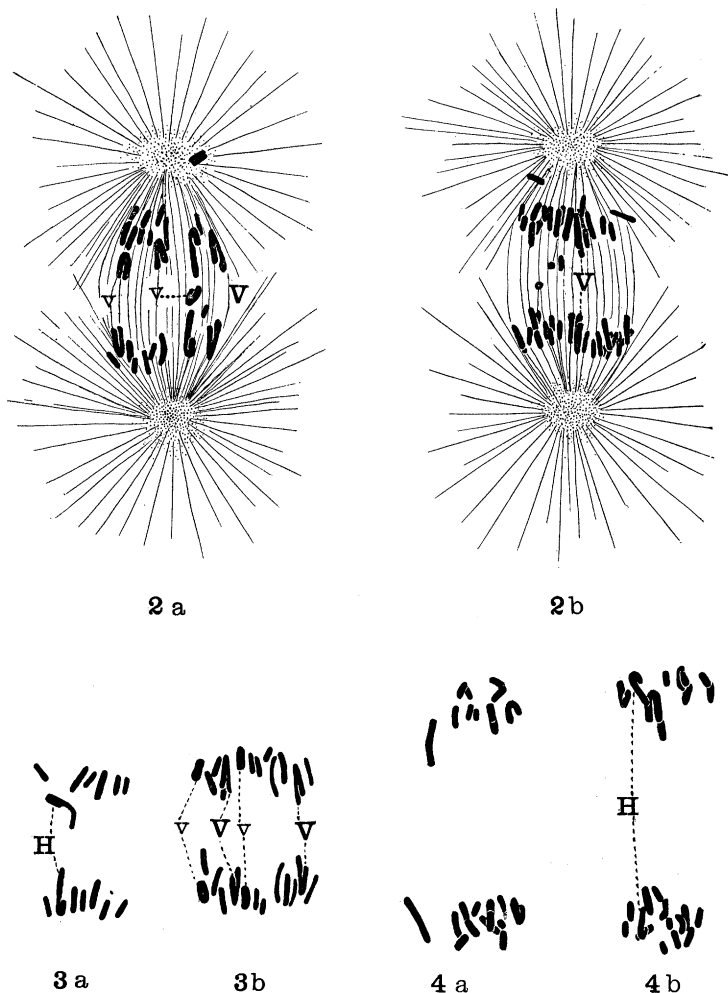
FIGS. 1a and 1b. *Hipponoë*. Chromosomes of equatorial plates from the same egg, two-celled stage. Drawn at a magnification of 1,500 diameters.

stages from a very unsafe basis for comparison. This is not meant to imply that length as a factor in identification is to be entirely eliminated.

In the later anaphases of *Hipponoë* three types of chromosomes appear. Commonest among these are the rod-shaped elements. As will be seen from Figs. 2a, 2b, 3a, 3b, 4a and 4b, none of these are of remarkable length. In Fig. 4a is given the only case that I found in which a long rod appeared. This unusual long chromosome would not resolve itself into two with the most careful focusing. Its isolated occurrence justifies the interpretation that it is an illustration of the tendency of *Hipponoë* chromosomes to clump together.

In none of my figures of *Hipponoë* are all of the rods shown. All attempts to separate the chromosomal complex into its units were unsuccessful. Figs. 2a and 2b show one spindle in which the chromosomes were unusually well separated. The variation in the thickness of the rods was reported by Heffner, '10, for *Toxopneustes*. Figs. 3a and 3b illustrate the same fact for *Hipponoë*. Accuracy is extremely difficult to achieve in the drawing of such minute objects as Echinoid chromosomes. All possible care was taken to indicate the true size relations.

Two somatic pairs of V's occur and these are shown in Figs. 5, 6, and 7 to be of two sizes. Their demonstration was not easy in the late division stages for as Miss Heffner found in *Toxopneustes*, the arms of the V's lie parallel. In *Hipponoë* one



FIGS. 2a and 2b. *Hipponoë*. One spindle in anaphase from two sections. Two pairs of V's and a hook are present.

FIGS. 3a and 3b. *Hipponoë*. Same as Figs. 2a and 2b. H, the hook-shaped chromosome.

FIGS. 4a and 4b. *Hipponoë*. Late anaphase. Not all of the rod-shaped chromosomes were drawn in these figures. All drawings made at a magnification of 1,500 diameters.



meets with the additional difficulty caused by the crowding of the chromosomes. For their study early anaphases proved most favorable. A reconstruction from one of these is shown in Fig. 6 and the V's from one are drawn in Fig. 7. The somatic pairs in these figures are easily recognized in this stage from their size as well as from their behavior. In the case of the pair of larger

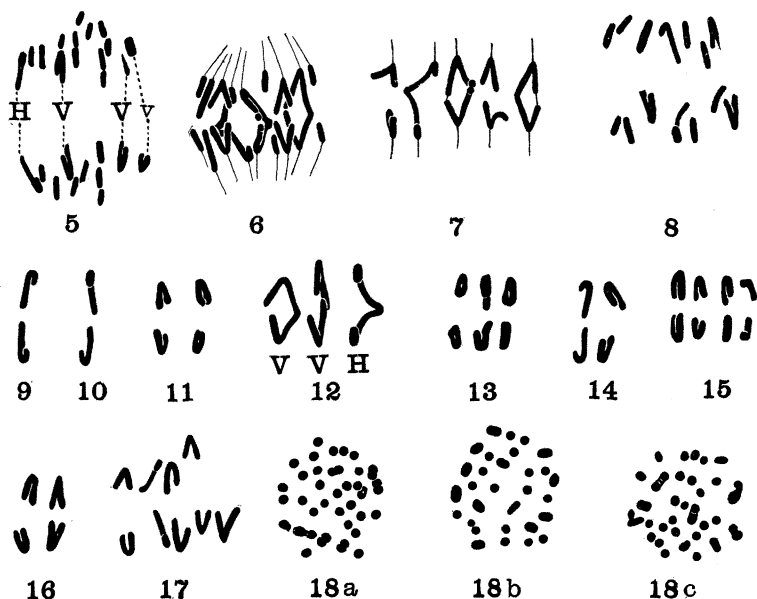


FIG. 5. *Hipponoë*. Mid anaphase.

FIG. 6. *Hipponoë*. Reconstruction from an early anaphase showing the relative size of the V's and hook.

FIG. 7. *Hipponoë*. V's and hook from one spindle.

FIG. 8. *Hipponoë*. Section showing large V's and hook.

FIGS. 9 to 17 inclusive. *Hipponoë*. The two-armed elements from nine different eggs. These figures illustrate the comparative frequency with which the different elements are observed. The hook is the most conspicuous. Fig. 15 is from an egg which contained no hook.

FIGS. 18a, 18b and 18c. *Hipponoë*. Polar views of daughter plates, 33, 30 and 32 chromosomes respectively. Magnification 1,500 diameters.

V's one arm of the V completes its division first in almost every instance. The question as to whether this is due to a mechanical hindrance in division or to a real difference in the size of the two arms has been discussed by Miss Heffner. I am inclined to the opinion that the two arms are of different lengths since that

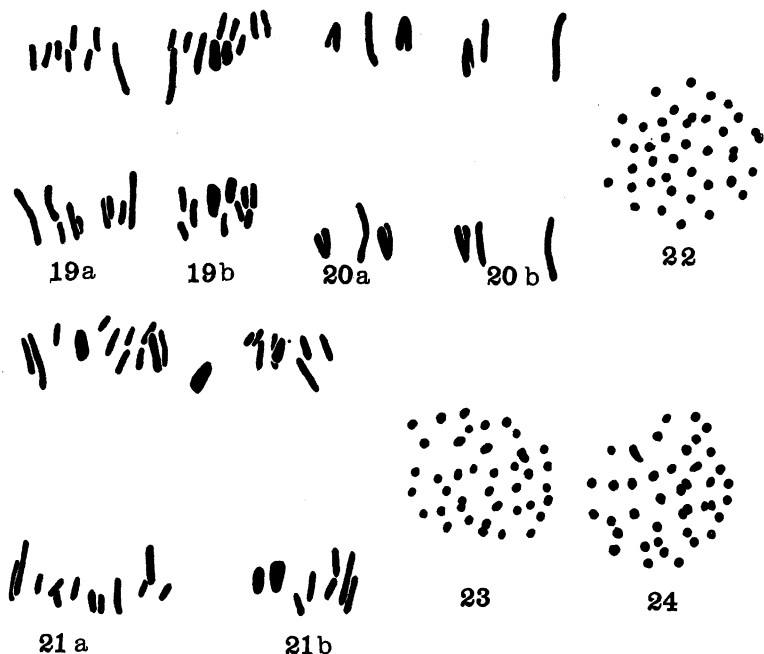
appearance predominates. Fig. 3*b* is a typical view. The larger pair is easily identified but one can be sure of only one smaller V. Its mate may be the small thick element, *v*, lying next to one of the larger V's. In Figs. 11, 12, 13, 15, 16 and 17 some of the V's from several other spindles are shown. Owing to individual differences in the eggs it would be impossible to identify the large or the small pair as such unless the other pair could be distinguished.

Miss Heffner did not observe any constant difference in size or behavior which might mark the unpaired V in *Toxopneustes*. The size relation of the *Hipponoë* V's is very plain in early anaphases as I have already explained.

I have examined sections of *Toxopneustes* eggs made from the same material that Miss Heffner used and am able to confirm her observations upon the appearance and behavior of the V-shaped elements. The long rods are also very conspicuous (Figs. 19, 20 and 21). A peculiarity, presumably of the egg, calls for different degrees of differentiation in staining early and late anaphases. I could not find on the slides that I examined early anaphases where the condition of things could be made out satisfactorily although there were plenty of later stages which were as clear as one could wish. In these I have observed and give figures (20 and 21) which show a difference in the size of the V's which would indicate that the unpaired V is the smallest, yet I hesitate to accept this alone as evidence that such is the case. Fig. 21 shows two large and one small V in the upper group. One large V is missing from the lower group. The indications were that it had been displaced by the knife in sectioning. Miss Heffner's Figs. 1*a*, and 3, *a* and *b*, depict two spindles in early anaphase. From these figures and from what I find in *Hipponoë* where such stages are comparatively abundant and convincing I feel justified in concluding that the unpaired V or heterochromosome in *Toxopneustes* is the smallest V of the complex.

In Figs. 3, 9 and 10 is shown the third type of echinoderm chromosome, the hook. It corresponds in all respects to the hook described by Baltzer. In *Hipponoë* it occurs singly and is not present in all of the eggs. Of twenty-nine eggs in which its presence or absence could be determined with certainty seven-

teen contained the hook. In late stages it is the most conspicuous element of the complex and is therefore quite easily detected when present; but when it is not found, one cannot always be sure that it is not hidden among the other chromosomes. In examining the eggs then more of those which lacked the hook would be rejected as positive evidence than of those in which it occurred, so I believe we are safe in asserting that half of the



FIGS. 19a and 19b. *Toxopneustes*. Late anaphase showing the long rods.

FIGS. 20a and 20b. *Toxopneustes*. Conspicuous chromosomes from a late anaphase.

FIGS. 21a and 21b. *Toxopneustes*. Late anaphase.

FIGS. 22, 23 and 24. *Toxopneustes*. Polar views of daughter plates, 38 chromosomes. Magnification 1,500 diameters.

fertilized eggs contain the hook and in half it is absent. As Dr. Tennent, '11, this journal, has shown, the dimorphism in somatic chromosome groups of fertilized *Hipponoë* eggs can be associated only with a dimorphism in the sperm.

The decision having been reached as to the differential character of the hook-shaped chromosome, the question arose as to

the nature of its morphological relation to the other chromosomes. All such elements of which we have microscopical proof, belong to two classes, the accessory or unpaired chromosomes and the paired heterochromosomes or idiochromosomes. This does not take into account those cases like *Culex* and *Theobaldia* where no differential element is visible in the sex cells. In both of these classes the germ cells of one sex lack a definite chromatin mass which is present in the other. The single or unequal pair of chromosomes of the one is replaced in the other by an equal pair both members of which correspond in size respectively to the *single element* or to the *larger element of the unequal pair*. In *Hipponoë* the search for evidence on this point revealed no pair of chromosomes equal to or exceeding the hook in size. This led to the conclusion that a corresponding pair of hooks does not exist. It was then thought that if such a pair was present its members were probably ordinary rods. No rods equal in size to the combined lengths of the two arms were present in any of the eggs. The question as to whether the hook was a multiple chromosome was considered. This probability which I will discuss later, suggested that perhaps the differential sex element consisted of only one arm of the hook. No rods equal in size to the long arm of the hook were discovered in any of the eggs. If such rods were present they might escape detection in many instances but it is improbable that they should be so consistently over-looked in all of the eggs. In Figs. 2*a* and 2*b* an attempt was made to draw all of the chromosomes from one spindle. The hook and V's are plainly visible. All of the elements shown could be separated by careful focusing but no rod longer than the arms of the large V's was observed. It appears highly probable that the long arm of the hook is unmated in somatic cells containing the hook and that no corresponding pair of long rods is present in cells which contain no hook. It would be impossible from this material to determine whether or not the short arm of the hook was mated. Many short rods approximating it in length occur but they could not be studied individually.

I have failed to determine the number of chromosomes in these eggs. Figs. 18*a*, 18*b* and 18*c* give some of the best polar views of daughter plates. Fig. 18*a* shows thirty-three bodies

one of which appears as though it might consist of two or more chromosomes crowded together. I was not able as were both Heffner and Baltzer to identify with certainty the V's or hook in such views. As these observers have explained, the ordinary rods seen in polar views appear as small black dots. Two of these in contact are to be interpreted as the closely appressed arms of the V-shaped chromosome. Miss Heffner's figures of the polar views of daughter groups of *Toxopneustes* chromosomes are very clear and her interpretation of them is undoubtedly correct. Baltzer also finds the V's in polar views appearing as two closely set dots. In polar views of *Hipponoë* I have often observed what I at first supposed might be a V. Besides the small round dots typical of the rod-shaped chromosomes, one finds in every plate a number of elliptical bodies of twice the size of the ordinary dots. These one naturally interprets as the two arms of a V with their adjacent surfaces flattened together. In some cases a very evident constriction coincident with their narrowest diameter makes such an interpretation imperative. This constriction is not however evident in the majority of cases and very often more of these elliptical bodies appear than are called for by the number of two-armed elements in *Hipponoë* (Fig. 18b). In all cases then the question is still open as to whether these are V's or two rod-shaped individuals or even, as is quite possible, only one rod viewed obliquely. The latter possibility in addition to the difficulty presented by untranslatable amorphous chromatin bodies in these daughter plates makes an accurate count impossible. The number counted most frequently was thirty-two but in no case could I feel sure that the number counted was correct.

*Moirā atropos.*

The egg of *Moirā* is much better suited to detailed study than the egg of *Hipponoë*. The tendency of the chromosomes to crowd together is not so prevalent. Although my material was very limited and contained comparatively few spindles some general facts were gathered which it may be worth while to record.

Fig. 25 is a polar view of the equatorial plate of the first segmentation nucleus. As already stated the chromosomes are

more slender than those of *Hipponoë*. Fig. 26 is a section through a metakinesis stage. Only a few of the chromosomes are shown. Three types appear; the ordinary rods, two small V's and two club-shaped elements. In Figs. 27 and 28, which are analytical drawings of two early *Moir*a spindles, the same three types appear. It could not be determined whether the club-shaped chromosomes

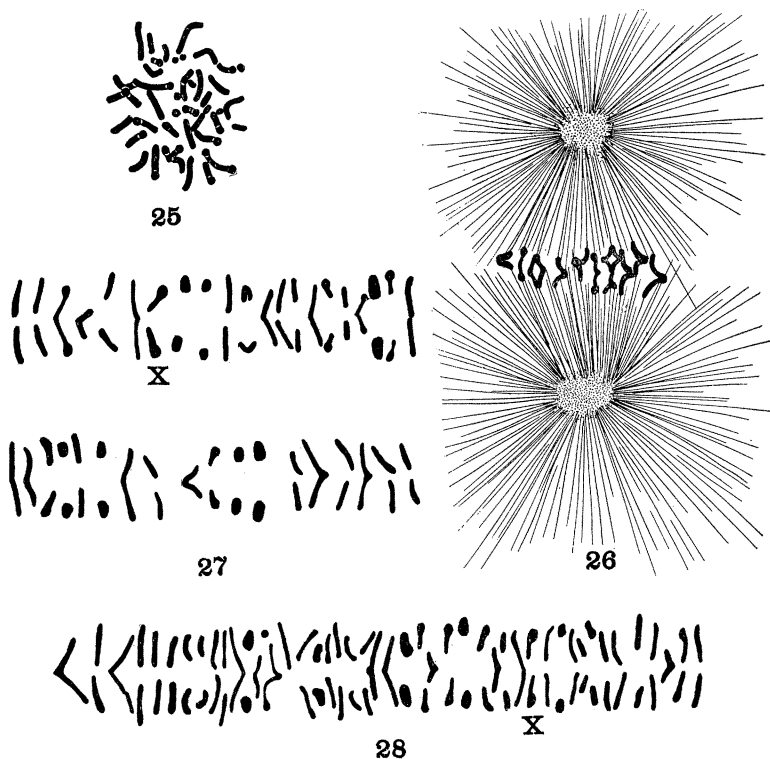


FIG. 25. *Moir*a. Equatorial plate from first segmentation nucleus.

FIG. 26. *Moir*a. Beginning anaphase.

FIG. 27. *Moir*a. Chromosomes from an early anaphase. X, hook?

FIG. 28. *Moir*a. Same as 27. Magnification, 1,500 diameters.

were small hooks or not. The chromosome X in each of the figures mentioned above has the appearance often presented by the *Hipponoë* hook. The small V's are numerous but their number is not evident. Although the *Moir*a chromosomes are comparatively well separated on the spindle the fact that the number is not the same in all of the figures is evidence that even

here mistakes in drawing are almost inevitable. It may be that some shown are really two chromosomes so close together that they could not be separated with the magnification used. The series is also taken from two or more sections and there is the ever present possibility that some may have been displaced. Figs. 29*a* and 29*b* show a later anaphase of which Fig. 30 is a detailed drawing. A hook (*x*) is shown to be present, but many more observations would be necessary to demonstrate its presence beyond a doubt. The club-shaped type of chromosome is not in evidence here.

As was to be expected from lateral views of anaphases the only daughter plates I found were especially adapted to an exact enumeration of their elements. The number is shown in Figs. 31*a* and 31*b*, which are daughter plates of the same spindle, to be forty-six, the largest number yet reported for the echinoderms. Fig. 31*b* was drawn from two sections. The character of the *Moir*a spindle is such that I consider the evidence as to the number of chromosomes gained from this one plate much more convincing than all of the combined evidence of a like sort which I obtained from *Hipponoë*. I hope to extend the observations on this species later.

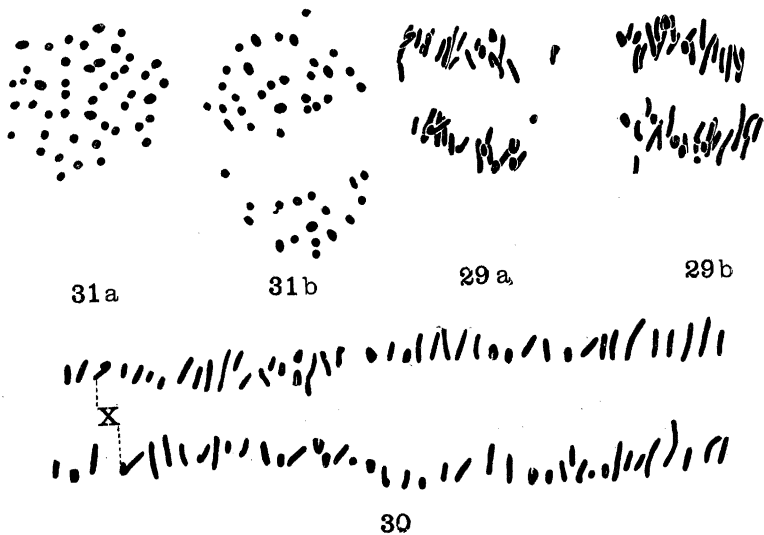
The *Moir*a material was collected at Beaufort, N. C., in 1907. Its treatment was identical with that of *Hipponoë*.

#### DISCUSSION.

From conclusions reached by Baltzer, '09, and Tennent, '11, it seems probable that in different species of a limited group the heterochromosome may originate in different sexes. Both of these authors used the same method of investigation so that the evidence of one bearing on this point possesses no peculiar advantage over that of the other. Both are equally justified in their conclusions. In *Strongylocentrotus lividus* and *Echinus microtuberculatus*, it is the female that is morphologically the heterogametic sex while the male is in the same sense homogametic. In the light of our present knowledge of the occurrence of the accessory chromosome in other groups these facts present an interesting anomaly.

If as Baltzer found in *Strongylocentrotus* and as Miss Heffner

found in *Toxopneustes* the somatic number of chromosomes is an even number in both sexes we must conclude that the odd chromosome is the eccentric member of an unequal pair. This of course implies the assumption that in the odd chromosome we are dealing with a univalent and not a plurivalent element. The number of chromosomes in *Hipponoë* may or may not be even. In the five species of sea-urchins already investigated the somatic number has been reported as even. The conclusions have however



FIGS. 29a and 29b. *Moira*. Anaphase from two sections.  
 FIG. 30. *Moira*. Chromosomes from Fig. 29. X, hook?  
 FIGS. 31a and 31b. *Moira*. Polar views of two daughter plates from the same spindle. 46 chromosomes. Fig. 31b is from two sections. 1,500 diameters.

been drawn from averages of many counts. Since the counts of single plates varied an exact statement as to whether the diploid number of chromosomes is the same and even in both sexes, or differs, being even in one and odd in the other, seems hardly justifiable.

In comparing daughter plates of *Hipponoë* with those of *Toxopneustes* I found as a rule that the chromosomes in *Toxopneustes* were better separated. Even there the majority of counts could not be made with any assurance of accuracy. Miss Heffner reported thirty-six chromosomes for *Toxopneustes*. I found four



plates which showed thirty-eight without any doubt. Three of them are shown in Figs. 22, 23 and 24. I also counted thirty-seven in two very clear daughter plates of the same cell.<sup>1</sup> I made a few counts where thirty-six seemed to be the number but in these cases I was not sure that I had counted correctly. Other counts were made but they were equally valueless. The *Toxopneustes* material was too limited to permit definite conclusions as to the actual numbers. I am convinced that more than thirty-six chromosomes occur in some eggs. An actual determination is difficult but not impossible. Averages in a case of this sort are useless.

Whatever the morphological relations of the hook to the other members of the complex, their consideration leads us to a recognition of possible existence of further anomalous conditions in this species. All unequal pairs of heterochromosomes or unpaired heterochromosomes of previous observation find a place in the series postulated by Stevens, 'II, which, beginning with *Culex* and *Theobaldia*, where no heterochromosome occurs, includes all of the various unequal pairs and ends with forms containing an odd or unpaired chromosome. We may imagine all of the members in this series derived from forms analogous to *Culex* by the gradual diminution and final elimination of one chromosome from a somatic pair. Assuming that the hook in *Hipponoë* is the morphological equivalent of the odd chromosome or the larger member of an unequal pair it follows that this element cannot be accounted for in this manner. On the contrary we must conceive of the change from the *Culex* heterochromosome type to the *Hipponoë* type to have come about by an increase in the size of one member of a somatic pair. It is quite as reasonable to suppose that the evolutionary processes within the nucleus involve an increase in the amount of chromatin as it is to assume that they are accompanied by its elimination. In support of this view we have the fact that the hook is the largest element in the *Hipponoë* complex.

<sup>1</sup> Prof. E. B. Wilson, in *Arch. f. Entw. d. Organismen v. Roux.*, Bd. XII., Taf. XVI., Fig. 43, gives a daughter plate of *Toxopneustes* showing 37 chromosomes. The explanation of the figure states that the number is 36. The coincidence is interesting in this connection. In earlier papers he raised the question as to whether the number was 36 or 38.

Baltzer has suggested that the hook is a plurivalent element composed of two chromosomes united at their polar ends. Admitting such an interpretation it still remains probable that *Hipponoë* cannot be included in the above series since it was impossible to find for the long arm of the hook of some eggs a corresponding pair of equal rods in others. The long rods of *Toxopneustes* are so conspicuous in late anaphases that it seems as though the presence of a similar pair in *Hipponoë* could not be a matter of dispute. Figs. 19, 20 and 21 show anaphases of *Toxopneustes* containing the characteristic long rods. These *Toxopneustes* rods are slightly longer than the long arm of the hook and the groups to which they belong are perhaps not so compact as daughter groups in *Hipponoë*.

With Baltzer's suggestion under consideration the question arises as to whether the V's which resemble the hook in all respects but length of arms are also to be regarded as multiple chromosomes. If so the method of grouping in *Hipponoë* differs from anything previously described.

The idea that the heterochromosome is associated with the inheritance of sex is a point of common consideration when treating of this differential element.

From the experimental evidence as to the inheritance of sex Castle, '09, has concluded that femaleness depends upon the presence of some factor wanting in the male, the differential factor. Two classes of female zygotes are recognized in Castle's scheme, one, class A in which "femaleness is attained only when the differential factor is doubly represented in the individual" and another, class B, in which femaleness is attained whenever the differential factor is present in one only of the conjugating gametes which produce the individual." The former for which Wilson gives the sex formulas; XX = female and X = male, X in this case designating the differential factor, is represented by the insects. Castle says, "Direct cytological evidence of the existence of class B is not known at present." The formulas, XO = female and O = male embody Castle's idea.

*Hipponoë* falls into class A only upon the assumption that the hook is a multiple chromosome, the long arm being the differential element, and that there exists in one-half of the fertilized eggs a

pair of rods the same length as the long arm. There is no observational basis as yet for either of these assumptions. Neither does our evidence place *Hipponoë* in class B since Professor Tennent's hybridization experiments show that the hook must come from the male. If however the hook is not a multiple chromosome the case is analogous to class B, the sex formulas being reversed to indicate that the male is a heterozygous dominant and the female a pure recessive,  $XO = \sigma$  and  $OO = \varphi$ . The conclusion is the same if we identify the differential element with the long arm of the hook and assume that none of the eggs contain a corresponding pair of long rods.

Clearly our conclusions as to the composition of the hook and its meaning must be deferred until our knowledge has been increased by further evidence gained from this and other species. McClung's views upon the taxonomic value of the chromosomal complex suggest that the study of other species of the same genus would prove most profitable. From a comparison of the two species of the genus *Arbacia* described by Tennent and Baltzer one finds similarities in the chromosomal groups which lend significance to this suggestion. Broader cytological study of the echinoderms is also necessary in order to discover the fundamental principle by which we can reconcile such diverse phenomena as those presented by *Strongylocentrotus* and *Echinus* on the one hand and *Hipponoë* on the other. The difficulties which attend the study of echinoderm chromosomes makes it desirable to extend the number of workers in this field.

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